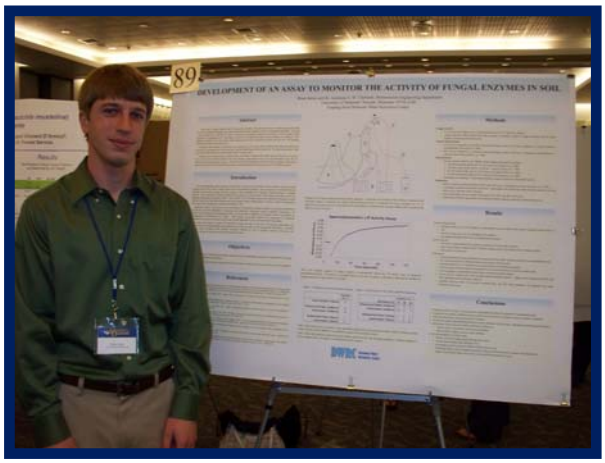
Report as of FY2009 for 2009DE153B: "Development of an Assay to Monitor the Activity of Fungal Enzymes in Soil"

Publications

- Other Publications:
  - Pautler, M., ed., 2009, Delaware Water Resources Center WATER NEWS Vol. 9 Issue 2 Introducing Our 2009-10 Spring Interns, http://ag.udel.edu/dwrc/newsletters/Winter08Spring09/WATERNEWSco-Spring2009.pdf , p. 5.
  - Pautler, M., ed., 2009, Delaware Water Resources Center WATER E-NEWS Vol. 8 Issue 5 Spotlight on 2009-10 DWRC Undergraduate Internships, http://ag.udel.edu/dwrc/newsletters/WATERENEWS-Oct2009.pdf , p. 2.
- Water Resources Research Institute Reports:
  - Jayne, B., and A. Chirnside, 2010, Development of an Assay to Monitor the Activity of Fungal Enzymes in Soil, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 8 pages.

Report Follows

Undergraduate Internship Project #2 of 10 for FY09



Intern ***Brian Jayne's*** project, sponsored by the ***DWRC***, was titled "Development of an Assay to Monitor the Activity of Fungal Enzymes in Soil." He was advised by Dr. Anastasia Chirnside of the ***UD's*** Department of Bioresources Engineering.

"Through my research, I developed an appreciation for organisms as seemingly simple as fungi for their abilities to aid us in some of our most complex environmental problems." – Brian Jayne

Abstract

The white rot fungus, *Phanerochaete chrysosporium*, produces non-specific enzymes, including lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP), that are able to degrade various environmental pollutants. The goal of this research was to develop an assay to monitor and accurately measure the activity of LiP and MnP during treatment of contaminated soil. The process consisted of growing the fungus in a packed-bed reactor and collecting the effluent, which contained extracellular enzymes. The effluent could be tested for enzyme activity using a continuous spectrophotometric assay of the oxidation of substrate. The enzyme solutions were added to dry soils and mixed. Various buffer solutions were then added to the soil samples to attempt to extract the enzymes back out of the soil. These buffer solutions included 100 mM phosphate buffer, 500 mM acetate buffer, 50 mM acetate buffer, and 50 mM bicarbonate buffer. These samples were centrifuged, and the liquid was analyzed using the spectrophotometric assay. During further experimentation, these centrifuged liquids were saturated to 80% with $(NH_4)_2SO_4$ to precipitate the enzymes and leave smaller interfering molecules dissolved. This purification step was used on both extracted buffer solutions and fresh extracellular enzyme solution.

Results were variable and indecisive throughout the research. None of the buffer solution extraction trials resulted in measurable enzyme activity. There were indications, however, that some activity may have been present at times, so extraction by treatment with a buffer solution may be a feasible practice. The purification steps with extracted buffer solutions also did not result in measurable enzyme activity. Purification steps on fresh extracellular enzyme solution that had not been added or extracted from soil demonstrated inconsistent but measurable enzyme activity. Activity was found in both redissolved pellet solution from $(NH_4)_2SO_4$ precipitation as well as the supernatant of this reaction. This suggests that some enzyme precipitation and interaction did occur; thus, the purification steps may also be feasible with further research. Several recommendations for future work are provided, such as proper cold storage of enzyme solutions, renewed fungus growth reactor, and reduced dilution of the enzymes in the soil from the addition of large buffer solution quantities.